

Three New Species of Dicyemid Mesozoans (Phylum Dicyemida) from *Amphioctopus fangsiao* (Mollusca: Cephalopoda), with Comments on the Occurrence Patterns of Dicyemids

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Three new species of dicyemid mesozoans are described from the renal appendages of *Amphioctopus fangsiao*, collected off Akashi, in Harima Nada, and from Osaka Bay. *Dicyema akashiense* n. sp. is a small species that reaches about 900 µm in length. The vermiform stages are characterized as having 15–17 peripheral cells, a conical calotte, and an axial cell that extends to the base of the metapolar cells. Infusoriform embryos consist of 37 cells; two nuclei are present in each urn cell, and the refringent bodies are solid. *Dicyema helocephalum* n. sp. is a small species that reaches about 800 µm in length. The vermiform stages are characterized as having 22 peripheral cells, a disc-shaped calotte, and an axial cell that extends to the base of the propolar cells. Infusoriform embryos consist of 37 cells; a single nucleus is present in each urn cell, and the refringent bodies are solid. *Dicyema awajiense* n. sp. is a small species that reaches about 300 µm in length. The vermiform stages are characterized as having 22 peripheral cells, a conical calotte, and an axial cell that extends to the middle of the propolar cells. Infusoriform embryos consist of 37 cells; a single nucleus is present in each urn cell, and the refringent bodies are solid.

In *A. fangsiao* various occurrence patterns of dicyemid species were observed, including instances where different dicyemid species were found in the renal appendage on each side. This suggests that dicyemids infect each renal appendage independently. The prevalence, reproductive traits, calotte shapes, and co-occurrence patterns of dicyemids are briefly discussed.

Key words: *Amphioctopus fangsiao*, cephalopods, convergence, dicyemids, infusoriform embryos, mesozoans, niche separation, parasites, renal organs

INTRODUCTION

Nouvel and Nakao (1938) published the first record of dicyemids in Japan. They described *Dicyema misakiense* Nouvel and Nakao, 1938 from *Octopus vulgaris* Lamarck, 1798, and *D. orientale* Nouvel and Nakao, 1938 from *Sepio-teuthis lessoniana* Lesson, 1830. Nouvel (1947) later described *D. acuticephalum* Nouvel, 1947 from *O. vulgaris* and identified a dicyemid species from *Sepia esculenta* Hoyle, 1885 as *Pseudicyema truncatum* Whitman, 1883, which had been described earlier in Europe. Subsequently, Furuya *et al.* (1992a) described two dicyemid species, *D. japonicum* Furuya and Tsuneki, 1992 and *D. clavatum* Furuya and Koshida, 1992, from *O. vulgaris* and *Callistoctopus minor* (Sasaki, 1920), respectively. Furuya (1999) later reported fourteen new species of dicyemid from six cephalopod species, *Amphioctopus fangsiao* (d'Orbigny, 1840), *C. minor*, *O. hongkongensis* Hoyle, 1885, *Enteroctopus doffeini* (Wülker, 1910), *S. esculenta*, and *S. lycidas* Gray, 1849, caught off the coasts of Japan. More recently, Furuya and Tsuneki (2005) described a new dicyemid species from *S. lessoniana*.

Nouvel (1947) reported dicyemids in the octopus host *Amphioctopus fangsiao*, noting the presence of *D. misakiense*, which also occurs in *O. vulgaris*. Subsequently, Furuya (1999) found five species of dicyemids from *A. fangsiao* and described two of them as *D. erythrum* and *D. colurum*. One of the other three species is probably Nouvel's *D. misakiense*. These three species were very similar to *D. acuticephalum*, *D. japonicum*, and *D. misakiense* in the vermiform stage, leading to the conclusion that *A. fangsiao* harbored two host-specific species and three species also infecting *O. vulgaris*. However, differences between the species infecting *A. fangsiao* and *O. vulgaris* are here recognized. In this paper, I describe three new species in the genus *Dicyema* from *A. fangsiao* collected off Akashi, in Harima Nada, and from Osaka Bay. In addition, I briefly discuss the occurrence patterns of dicyemid species in *A. fangsiao*.

MATERIALS AND METHODS

In this study, 507 individuals of *Amphioctopus fangsiao* were examined for dicyemids from December 1989 to April 2005. Host specimens were obtained from fishermen. When dicyemids were detected in the renal organ of a host cephalopod, small pieces of the renal organ with attached dicyemids were removed and smeared on glass microscope slides. The smears were fixed immediately in Bouin's fluid for 24 hr and then stored in 70% ethyl alcohol. Most of them were stained in Ehrlich's hematoxylin and coun-

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terstained in eosin. Stained smears were mounted with Entellan (Merck). Dicyemids were observed with a light microscope (Olympus BH-2) at magnifications up to 2000 \times . Measurements and drawings were made with the aid of an ocular micrometer and a drawing tube (Olympus U-DA), respectively.

The renal organs were also fixed with Bouin's fluid. The fixed organs were embedded in paraffin and sectioned transversely. The sections were stained with hematoxylin-eosin and observed histologically.

The terminology for cell names used in the description of infusoriform larvae is based on Nouvel (1948), Short and Damian (1966), Furuya *et al.* (1992b, 1997), and Furuya (1999).

Syntypes of the dicyemids are deposited in the Osaka University Museum, Toyonaka, Osaka, Japan (OUM), the Santa Barbara Museum of Natural History, Santa Barbara, California, USA (SBMNH), and in the author's collection. The symbiotypes (octopus specimens harboring the dicyemid types) are deposited in the OUM.

RESULTS

Histology of the renal organ in *Amphioctopus fangsiao*

The octopus kidney consists of a pair of sac-like renal organs, each containing a light yellow renal appendage. *Amphioctopus fangsiao* usually has red-colored renal organs; the color is attributable to the presence of *D. erythrum*, the dominant dicyemid species in *A. fangsiao*. This dicyemid species is characterized by red granules within the cytoplasm of the peripheral cells (Furuya, 1999). Occasionally, one renal organ is yellowish (Fig. 1a, b), and may or may not contain *D. erythrum*. The other dicyemid species are colorless because they possess few red granules in their peripheral cells.

The renal appendages are much folded and consist of a single-layered microvillous columnar epithelium covering the vena cava and its branches (Fig. 1c–e). Dicyemids with cone-shaped calottes, such as *D. akashiense* sp. nov., *D. awajense* sp. nov., and *D. erythrum*, insert the anterior part of the body into folds or crypts in the renal appendages (Fig. 1c–e). In contrast, dicyemids with a slightly inflated, disc-shaped calotte, such as *D. helocephalum* sp. nov., are attached to the surface of the renal appendages (Fig. 1c).

Taxonomy

Dicyemidae van Beneden, 1882

Dicyema von K  lliker, 1849

Dicyema akashiense sp. nov.

(Figs. 1–3; Table 1)

Diagnosis. Small-sized dicyemids, body length typically not exceeding 900 μ m. Peripheral cell number of vermiform stages (*i.e.*, vermiform embryo, nematogen, and rhombogen) 14–17, typically 16: 4 propolars, 4 metapolars, and 6–9 trunk cells. Calotte relatively large, conical. Infusoriform embryos consisting of 37 cells; urn cells with 2 nuclei each.

Type series. Syntypes: OUM-ME-00006 (1 slide); SBMNH-358803 (1 slide). Additional syntypes on slide series No. HF-FA114 (5 slides, author's collection).

Type locality. Japan, Hyogo Prefecture, Osaka Bay, Off Akashi, 34°37'N, 135°02'E. Date of collection, 21 May 1991.

Etymology. The specific name refers to the type locality, expressed as a Latinized adjective in the nominative case agreeing in gender (neuter) with the genus name.

Symbiotype. *Amphioctopus fangsiao* (Cephalopoda:

Octopoda: Octopodidae), mature male, 42 mm ML. OUM-MO-00005 (author's code FA114).

Site of infection. Within renal sacs; anterior ends (calottes) inserted into crypts of the renal appendages (Fig. 1c).

Incidence. Dicyemids found in 124 host octopuses, obtained in Harima Nada and Osaka Bay: 24.5% occurrence among 507 octopuses examined.

Distribution. Known only from the type locality.

Description. *Nematogens* (Fig. 2a, b): Body slender; lengths ranging from 300 to 900 μ m, widths from 30 to 60 μ m. Peripheral cell number 15–17, typically 16 (Table 1): 4 propolars, 4 metapolars, 2 parapolars, 3–5 diapolars, and 2 uropolars. Calotte bluntly rounded, conical (Figs. 2b; 3a, d). Cilia on calotte short, about 4 μ m long, oriented forward. Cytoplasm of both propolar and metapolar cells stained with hematoxylin. Propolar cells and their nuclei smaller than metapolar cells and their nuclei, respectively. Trunk mostly uniform in width. Trunk cells arranged in opposed pairs. Occasionally trunk cells with many granular inclusions (Fig. 2b). Axial cell cylindrical, rounded anteriorly, extending forward to base of metapolar cells (Fig. 2b). In axial cell of large individuals, 15–30 vermiform embryos present. Accessory nuclei seen in trunk peripheral cells.

Vermiform embryos (Figs. 2d; 3f, g): Full-grown vermiform embryos small-sized; length ranging from 30 to 60 μ m, width from 7 to 12 μ m; peripheral cell number 16 or 17 (Table 1). Anterior end of calotte tapered anteriorly, bluntly rounded. Trunk cells arranged in opposed pairs. Axial cell rounded anteriorly, extending forward to middle of metapolar cells (Figs. 2d; 3f). Axial cell nucleus typically located in center of axial cell. Axial cell of full-grown embryos containing 2–6 agametes.

Rhombogens (Figs. 2a, c; 3b, c, e): Slightly stockier than nematogens, otherwise generally similar in shape and body proportions; length ranges from 300 to 900 μ m; width from 40 to 60 μ m. Peripheral cell number 14–16, typically 16 (Table 1). Calotte conical as in nematogens. Shape and anterior extent of axial cell similar to those of nematogens. Number of infusorigens present in axial cell 1, rarely 2. In the axial cell of large individuals, 5–10 infusoriform embryos typically present. Accessory nuclei present in peripheral cells.

Infusorigens (Fig. 2e): Small-sized. Axial cell of infusorigens usually rounded, diameter 9–12 μ m. In mature infusorigens ($n=20$), number of external cells (oogonia and primary oocytes) 6–12 (mode, 8), number of internal cells (spermatogonia, primary spermatocytes, and secondary spermatocytes) 3–6 (mode, 4), and number of sperm 4–14 (mode, 7). Diameter of fertilized eggs 10.2 μ m; diameter of sperm 1.9 μ m.

Infusoriform embryos (Figs. 2f, g; 3h–j): Ovoid, bluntly pointed posteriorly. In full-grown embryos ($n=50$), length (excluding cilia) 26.2 ± 1.7 μ m (mean \pm S.D.); length-width-height ratio 1:0.74:0.71. Cilia at posterior end, 6.9 μ m long. Refracting bodies present, solid, occupying anterior 70% of embryo length when viewed laterally (Fig. 2g). Nuclei of apical internal cells usually seen between refracting bodies (Fig. 2g). Cilia projecting from ventral internal cells into urn cavity (Fig. 3j). Cytoplasm of dorsal internal cells transparent. Capsule cells with many minute granular inclusions. Full-grown infusoriform embryos ($n=50$) consisting of 37 cells: 33 somatic and 4 germinal cells. Somatic cells of sev-

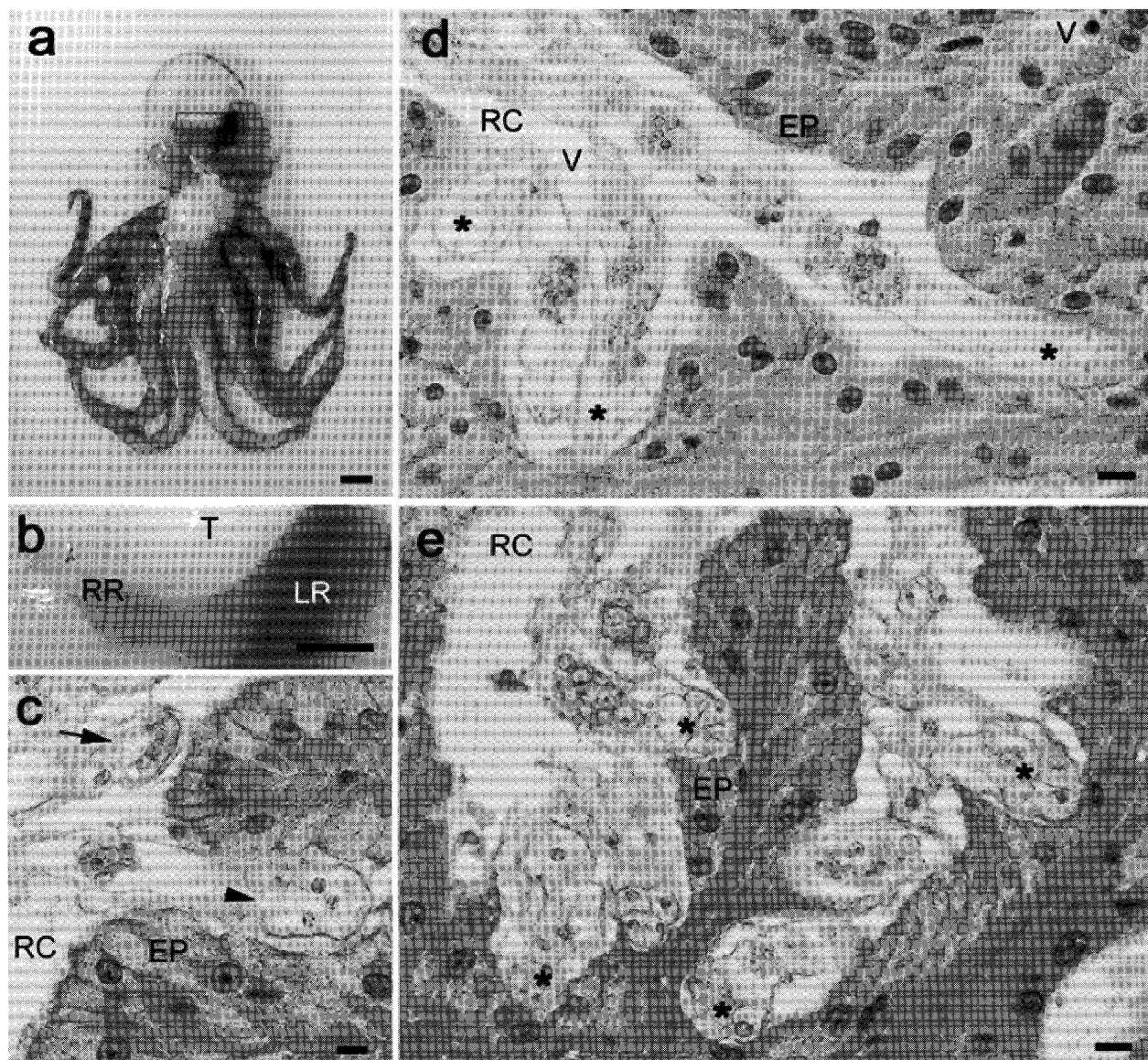


Fig. 1. **a**, *Amphioctopus fangsiao* (ventral side); **b**, the region indicated by a rectangle in Fig. 1a; **c–e**, light micrographs of stained sections through the renal organ: **c**, *Dicyema akashiense* (arrowhead), *D. helocephalum* (arrow); **d**, *Dicyema erythrum* (asterisk); **e**, *Dicyema awajiense* (asterisk). Scale bars represent 10 mm in **a** and **b**, 10 μ m in **c–e**. EP, epithelial cell of renal appendage; LR, left renal sac; RC, renal coelom; RR, right renal sac; T, testis; V, vein.

eral types: external cells covering large part of anterior and lateral surfaces of embryo (2 enveloping cells); external cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, 2 posteroventral lateral cells); external cells with refringent bodies (2 apical cells); external cells without cilia (2 first ventral cells, 2 second ventral cells, 1 couvicle cell); internal cells with cilia (2 ventral internal cells); and internal cells without cilia (2 apical internal cells, 2 dorsal internal cells, 2 capsule cells, 4 urn cells). Each urn cell containing 1 germinal cell and 2 nuclei (Fig. 3j). All somatic nuclei typically become pycnotic as infusoriform embryos mature.

Remarks. *Dicyema akashiense* sp. nov. has been found together with *D. erythrum* and *D. colurum* in *Amphioctopus*

fangsiao. It is easily distinguishable from *D. erythrum* by the number of peripheral cells of vermiform stages (15–17 vs. 32–36). It is also easily distinguishable from *D. colurum* by the number of peripheral cells (15–17 vs. 22) and the calotte shape of vermiform stages (cone-shaped vs. disc-shaped).

Dicyema akashiense is very similar to *D. acuticephalum* in the calotte shape of vermiform stages and the cellular composition and cell number of infusoriform embryos (Furuya *et al.*, 1997; Furuya *et al.*, 2004a). Vermiform stages of *D. acuticephalum* have 16–18 peripheral cells, typically 18 (Nouvel, 1947; Furuya *et al.*, 1992a). In *D. akashiense*, however, no individuals have 18 peripheral cells. A few individuals with 16 or 17 peripheral cells occur in both species (Fig. 4). The maximum number of peripheral cells is 16 and 18 in *D. akashiense* and *D. acuticephalum*, respec-

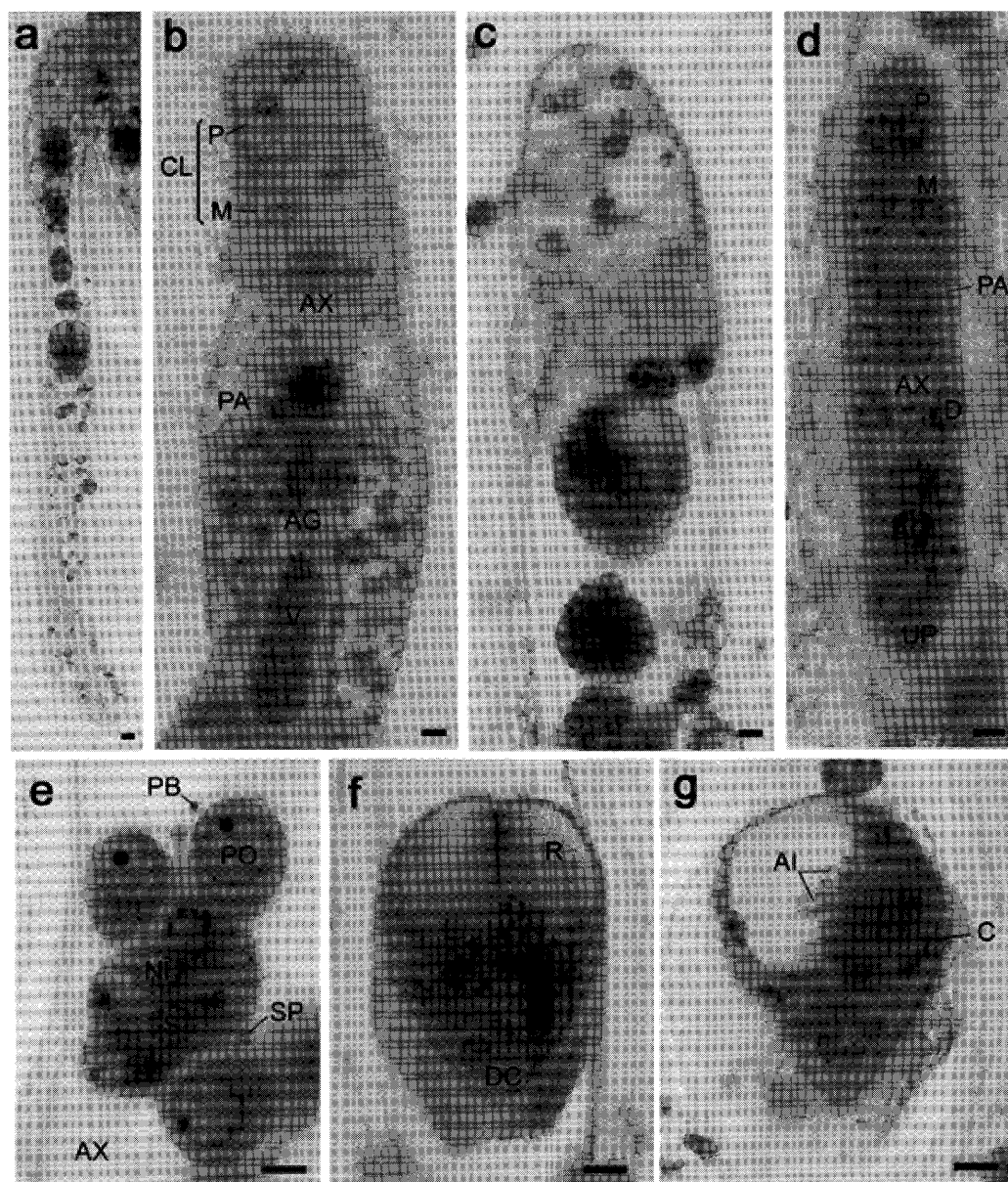


Fig. 2. *Dicyema akashiense* sp. nov. Photographs from syntype specimens on slide OUM-ME-00006. **a**, rhombogen, entire; **b**, nematogen, anterior region; **c**, rhombogen, anterior region; **d**, vermiform embryo within axial cell; **e**, infusorigen; **f**, **g**, infusoriform embryo; **f**, horizontal section; **g**, sagittal section. Scale bars represent 5 μ m. AG, agamete (axoblast); AL, apical internal cell; AX, axial cell; C, couvercle cell; CL, calotte; D, diapolar cell; DC, dorsal caudal cell; I, infusoriform embryo; M, metapolar cell; NI, nucleus of axial cell of infusorigen; P, propolar cell; PA, parapolar cell; PB, polar body; PO, primary oocyte; R, refrangent body; S, spermatogonium; SP, sperm; U, urn cell; UP, uropolar cell; V, vermiform embryo.

tively. Infusoriform embryos are larger in *D. acuticephalum* than in *D. akashiense* ($29.8 \pm 2.2 \mu\text{m}$ vs. $26.2 \pm 1.7 \mu\text{m}$). In addition, the axial cell of full-grown vermiform embryos of *D. akashiense* contains 4–6 agametes, whereas that of *D. acuticephalum* contains at most 4 agametes.

Dicyema akashiense is also similar to *D. bilobum* Couch and Short, 1964, *D. knoxi* Short, 1971, *D. typus* van Beneden, 1876, and *D. typoides* Short, 1964 in calotte shape and the number of peripheral cells. The infusoriform embryos of *D. akashiense* consist of 37 cells and are easily distinguished from those of *D. typus* (35) and *D. typoides* (35). The infusoriform embryos of *D. bilobum* and *D. knoxi* also

consist of 37 cells. However, *D. akashiense* differs from these two species in the cellular composition of infusoriform embryos. The infusoriform embryos of *D. bilobum* have third ventral cells, and those of *D. knoxi* have postcapsular cells instead of apical internal cells.

***Dicyema helocephalum* sp. nov.**

(Figs. 1, 5, 6; Table 2)

Diagnosis. Small-sized dicyemids, body length typically not exceeding 800 μm . Peripheral cell number of vermiform stages (*i.e.*, vermiform embryo, nematogen, and rhombogen) 22: 4 propolars, 4 metapolars, 2 parapolars, and 12

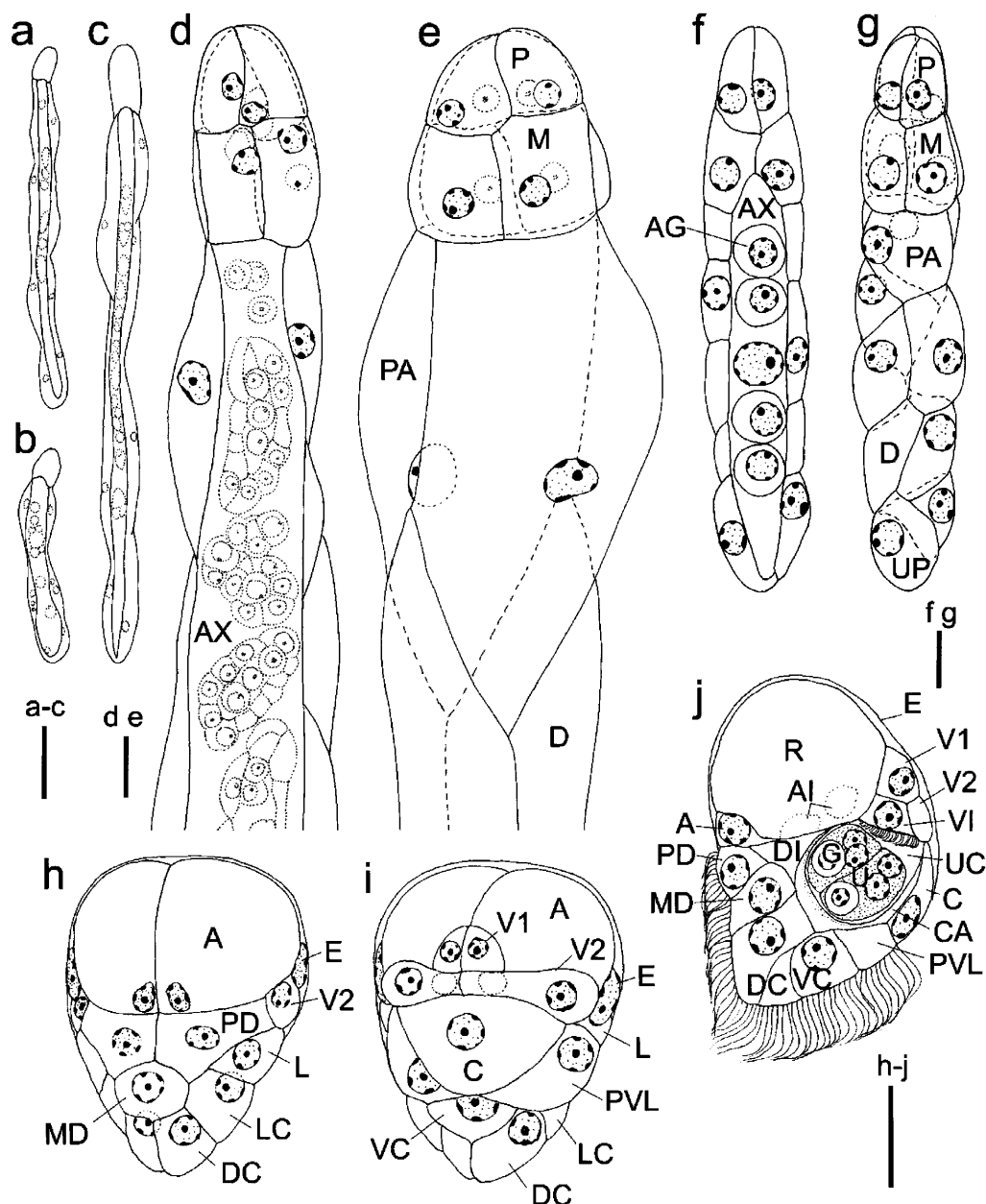


Fig. 3. *Dicyema akashiense* sp. nov. Drawn from syntype specimens on slide OUM-ME-00006. **a**, nematogen, entire; **b**, **c**, rhombogen, entire; **d**, nematogen, anterior region; **e**, rhombogen, anterior region; **f**, **g**, vermiform embryos within the axial cell (cilia omitted); **f**, optical section; **h–j**, infusoriform embryos; **h**, dorsal view (cilia omitted); **i**, ventral view (cilia omitted); **j**, sagittal section. Scale bars represent 5 μ m in **a–c** and 10 μ m in **d–j**. A, apical cell; AG, agamete (axoblast); AI, apical internal cell; AX, axial cell; C, couvercle cell; CA, capsule cell; D, diapolar cell; DC, dorsal caudal cell; DI, dorsal internal cell; E, enveloping cell; G, germinal cell; L, lateral cell; LC, lateral caudal cell; M, metapolar cell; MD, median dorsal cell; P, propolar cell; PA, parapolar cell; PD, paired dorsal cell; PVL, posteroventral lateral cell; R, refringent body; U, urn cell; UC, urn cavity; UP, uropolar cells; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V2, second ventral cell.

Table 1. *Dicyema akashiense* sp. nov.: number of peripheral cells.

Cell number	Number of individuals		
	Vermiform embryos	nematogens	rhombogens
14	0	0	1
15	0	2	4
16	46	47	45
17	4	1	0

trunk cells. Calotte disc-shaped; cephalic swelling formed together with parapolar cells. Infusoriform embryos consisting of 37 cells; urn cells with single nucleus each.

Type series. Syntypes: OUM-ME-00007 (1 slide); SBMNH-358804 (1 slide). Additional syntypes on slide series No. HF-FA1141 (5 slides, author's collection).

Type locality. Japan, Hyogo Prefecture, Osaka Bay, Off Akashi, 34°37'N, 135°02'E. Date of collection, 19 January 2005.

Etymology. From the Greek words *hel*, meaning "pin",

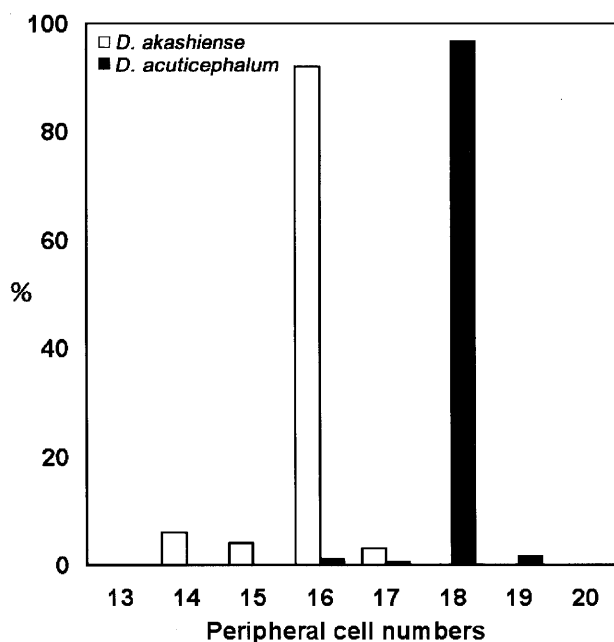


Fig. 4. Frequency distribution of peripheral cell numbers in vermiform individuals of *D. akashiense* and *D. acuticephalum*. (n=150 and 182 in the two species, respectively).

and *cephalos*, meaning "head", in reference to the characteristic pin-shaped calotte or head in adult stages.

Symbiotype. *Amphioctopus fangsiao* (Cephalopoda: Octopoda: Octopodidae), mature female, 61 mm ML. OUM-MO-00006 (author's code FA1141).

Site of infection. Within renal sacs; anterior ends (calottes) attached to the surface of renal appendages (Fig. 1c).

Incidence. Dicyemids found in 34 host cephalopods obtained in Harima Nada and Osaka Bay: 6.7% occurrence among 507 octopuses examined.

Distribution. Known only from type locality.

Description. *Nematogens* (Figs. 5a, c; 6a, c): Body hammer-like; length ranging from 200 to 800 μm ; width from 30 to 60 μm . Peripheral cell number 22 (Table 2): 4 propolars, 4 metapolars, 2 parapolars, 10 diapolars, and 2 uropolars. Calotte disc-shaped (Figs. 5c; 6c). Cilia on calotte about 4 μm long, oriented forward. Cytoplasm of propolar and metapolar cells conspicuously stained with hematoxylin trunk cells (Fig. 5a, c). Propolar cells and their nuclei smaller than metapolar cells and their nuclei, respectively. Axial cell cylindrical, rounded anteriorly, extending forward to base of propolar cells (Fig. 5c). About 30 vermiform embryos typically present in axial cell of large individuals. Uropolar cells occasionally verruciform.

Vermiform embryos (Figs. 5d; 6e, f): Full-grown vermiform embryos small-sized; length ranging from 40 to 60 μm , width from 9 to 12 μm ; peripheral cell number 22 (Table 2). Anterior end of calotte rounded. Trunk cells arranged in opposed pairs. Axial cell tapered anteriorly, extending forward to base of propolar cells, as in nematogens (Fig. 5d). Axial cell nucleus usually located in center or occasionally in anterior half of axial cell. Axial cell of full-grown embryos typically contains 1 or 2 agametes.

Rhombogens (Figs. 5b; e; 6b, d): Slightly stockier than

nematogens, otherwise generally similar to them in shape and body proportions; length ranges from 200 to 600 μm ; width from 30 to 60 μm . Peripheral cell number 22 (Table 2). Cephalic enlargement composed of calotte and parapolar cells, as in nematogens. Calotte disc-shaped (Fig. 5e). Shape and anterior extent of axial cell similar to those of nematogens. Number of infusorigens present in axial cell of rhombogen either 1 or 2. In axial cell of large individuals, 10–20 infusoriform embryos typically present. Accessory nuclei occasionally observed in trunk peripheral cells. Uropolar cells occasionally verruciform (Fig. 5b).

Infusorigens (Fig. 5f): Small-sized. Axial cell of infusorigens usually rounded, diameter 7–10 μm . In mature infusorigens (n=20), number of external cells (oogonia and primary oocytes) 7–15 (mode, 8), number of internal cells (spermatogonia, primary and secondary spermatocytes) 3–5 (mode, 4), and number of sperm 4–12 (mode, 8). Diameter of fertilized eggs 7.8 μm ; diameter of sperm 1.2 μm .

Infusoriform embryos (Figs. 5g, h; 6g–i): Ovoid, rounded to bluntly pointed posteriorly. In full-grown embryos (n=50), length (excluding cilia) 18.3 ± 0.8 μm (mean \pm S.D.); length-width-height ratio 1:0.76:0.73. Cilia at posterior end 4.4 μm long. Refrangent bodies present, solid; about the same size as two urn cells, occupying about 30–40% of embryo length when viewed laterally (Fig. 5h). Cilia projecting from ventral internal cells into urn cavity (Fig. 6i). Cytoplasm of dorsal internal cells transparent (Fig. 5h). Capsule cells containing many large granules (Figs. 5h; 6i). Full-grown infusoriform embryos (n=50) consisting of 37 cells: 33 somatic and 4 germinal cells. Somatic cells of several types: external cells covering large part of anterior and lateral surfaces of embryo (2 enveloping cells); external cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, 2 posteroventral lateral cells); external cells with refrangent bodies (2 apical cells); external cells without cilia (2 first ventral cells, 2 second ventral cells, 2 third ventral cells, 1 covercle cell); internal cells with cilia (2 ventral internal cells); and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, 4 urn cells). Each urn cell containing 1 germinal cell and 1 nucleus (Fig. 6i). Nuclei of third ventral cells pycnotic. All somatic nuclei becoming pycnotic as infusoriform embryos mature.

Remarks. *Dicyema helocephalum* sp. nov. has been found together with *D. akashiense* and *D. erythrum* in *Amphioctopus fangsiao*. It is easily distinguishable from *D. akashiense* and *D. erythrum* by calotte shape (disc-shaped vs. cone-shaped) and the number of peripheral cells of vermiform stages (22 vs. 15–17 and 32–36, respectively). *Dicyema colurum* also is found in *A. fangsiao*, but it has never been found together with *D. helocephalum*. *Dicyema colurum* is similar to *D. helocephalum* in calotte shape, number of peripheral cells in vermiform stages, and number of cells in infusoriform embryos, but it differs from the latter in having third ventral cells instead of anterior lateral cells, and in having two nuclei in the urn cells in infusoriform embryos.

Dicyema helocephalum is very similar to *D. japonicum*, *D. sphyrocephalum* Furuya, 1999, and *D. orientale* in calotte shape, number of peripheral cells in vermiform stages, and number of cells in infusoriform embryos. However, it can be distinguished from *D. japonicum* by the much smaller body

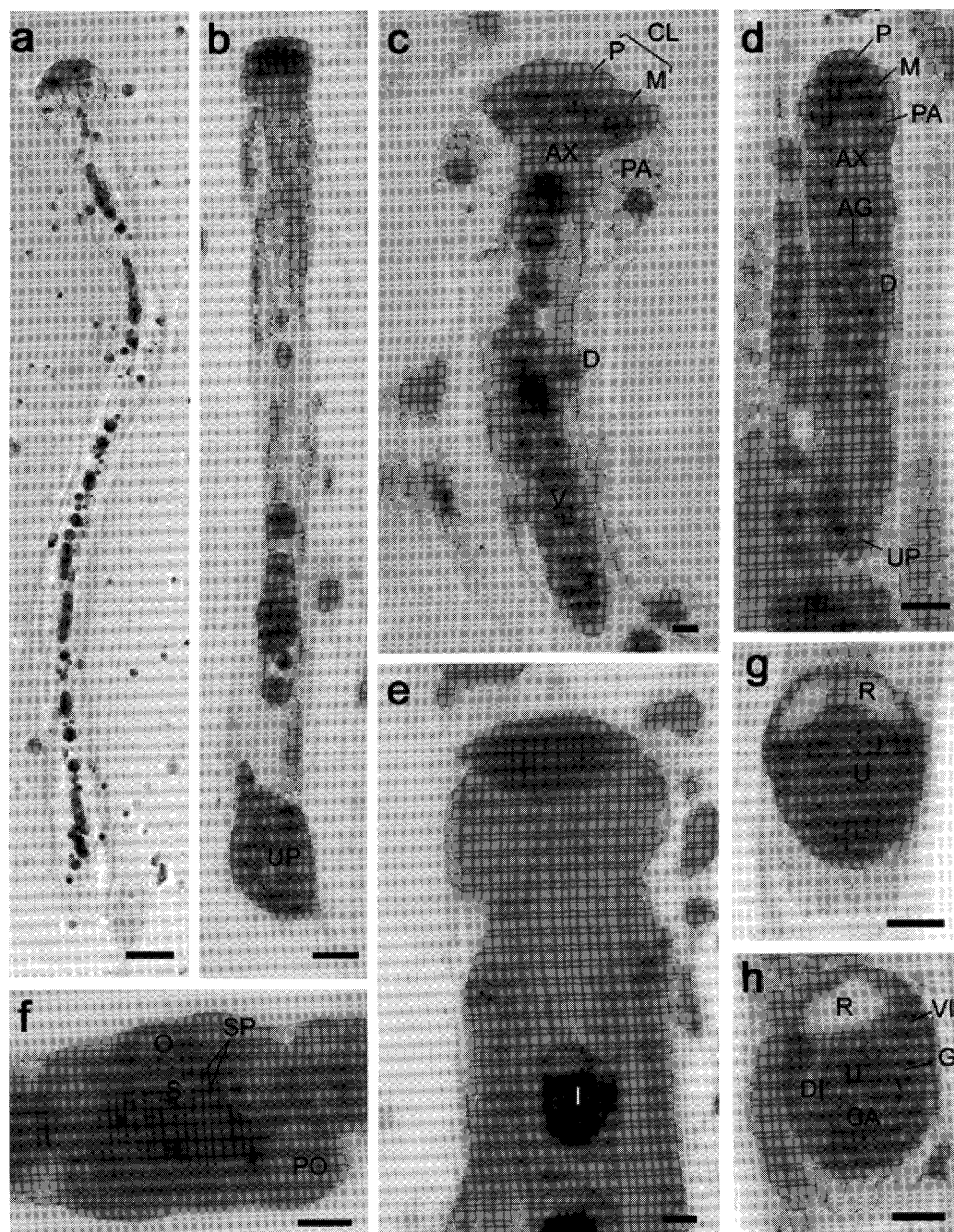


Fig. 5. *Dicyema helocephalum* sp. nov. Photographs from syntype specimens on slide OUM-ME-00007. **a**, nematogen, entire; **b**, rhombogen, entire; **c**, nematogen, anterior region; **d**, vermiform embryo within axial cell; **e**, rhombogen, anterior region; **f**, infusorigen; **g**, **h**, infusoriform embryo; **g**, horizontal section; **h**, sagittal section. Scale bars represent 20 μ m for **a** and **b**, 5 μ m for **c**–**h**. AG, agamete (axoblast); AX, axial cell; CA, capsule cell; CL, calotte; D, diapolar cell; DI, dorsal internal cell; G, germinal cell; I, infusoriform embryo; M, metapolar cell; O, oogonium; P, propolar cell; PA, parapolar cell; PO, primary oocyte; R, refrigrant body; S, spermatogonium; SP, sperm; U, urn cell; UP, uropolar cells, V, vermiform embryo; VI, ventral internal cell.

size of full-grown vermiform stages (800 μ m vs. 1800 μ m) and by having third ventral cells instead of anterior lateral cells in infusoriform embryos. *Dicyema helocephalum* differs from *D. sphyrocephalum* in having a stockier body in the full-grown vermiform stages and in having third ventral cells instead of anterior lateral cells in infusoriform embryos. It differs from *D. orientale* in having a much smaller body size of full-grown vermiform stages (800 μ m vs. 5000 μ m), third ventral cells instead of anterior lateral cells, and a single nucleus in each urn cell.

Dicyema benthoctopi Hochberg and Short, 1970, also is

similar to *D. helocephalum* in calotte shape and the mean number of peripheral cells, but the two species are distinguishable by the constancy of peripheral cell number in *D. helocephalum* (22, compared to 17–23, in *D. benthoctopi*) and the size of the full-grown vermiform stages (larger in *D. benthoctopi*).

***Dicyema awajiense* sp. nov.**

(Figs. 7, 8; Table 3)

Diagnosis. Small-sized dicyemids, body length typically not exceeding 400 μ m. Peripheral cell number of vermiform

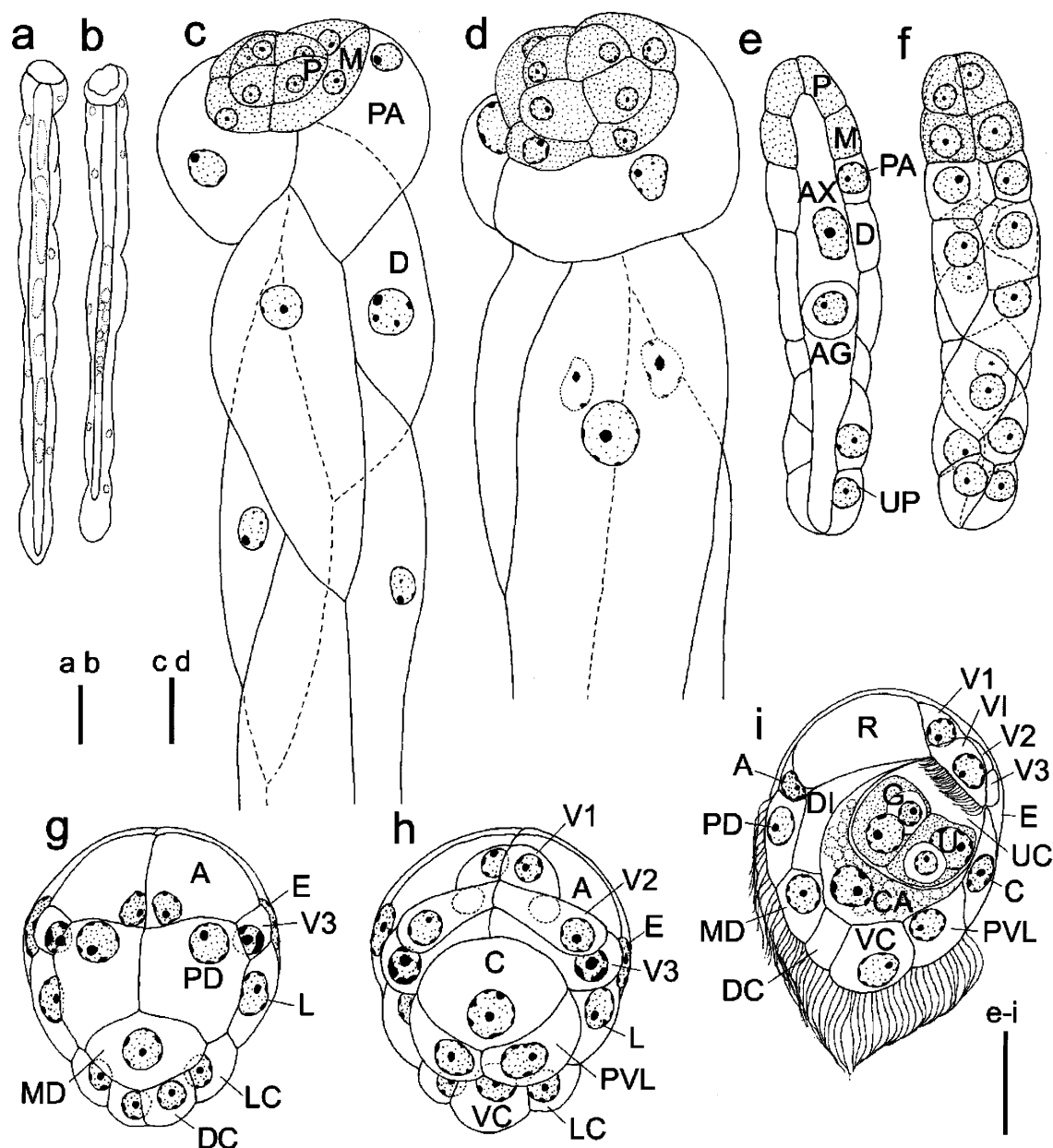


Fig. 6. *Dicyema helocephalum* sp. nov. Drawn from syntype specimens on slide OUM-ME-00007. **a**, nematogen, entire; **b**, rhombogen, entire; **c**, nematogen, anterior region; **d**, rhombogen, anterior region; **e**, **f**, vermiform embryos within the axial cell; **e**, optical section; **g–i**, infusoriform embryos; **g**, dorsal view (cilia omitted); **h**, ventral view (cilia omitted); **i**, sagittal section. Scale bars represent 50 μ m for **a** and **b**, 10 μ m for **c–i**. A, apical cell; AG, agamete (axoblast); AX, axial cell; C, couvercle cell; CA, capsule cell; D, diapolar cell; DC, dorsal caudal cell; DI, dorsal internal cell; E, enveloping cell; G, germinal cell; L, lateral cell; LC, lateral caudal cell; M, metapolar cell; MD, median dorsal cell; P, propolar cell; PA, parapolar cell; PD, paired dorsal cell; PVL, posteroventral lateral cell; R, refringent body; U, urn cell; UC, urn cavity; UP, uropolar cells; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V2, second ventral cell; V3, third ventral cell.

Table 2. *Dicyema helocephalum* sp. nov.: number of peripheral cells.

Cell number	Number of individuals		
	Vermiform embryos	nematogens	rhombogens
22	50	50	50

stages (*i.e.*, vermiform embryo, nematogen, and rhombogen) 22: 4 propolars, 4 metapolars, and 14 trunk cells. Calotte relatively small, conical. Infusoriform embryos consisting of 37 cells; urn cells with single nucleus each.

Type series. Syntypes: OUM-ME-00008 (1 slide); SBMNH-358805 (1 slide). Additional syntypes on slide series No. HF-FA1260 (5 slides, author's collection).

Type locality. Japan, Hyogo Prefecture, Osaka Bay,

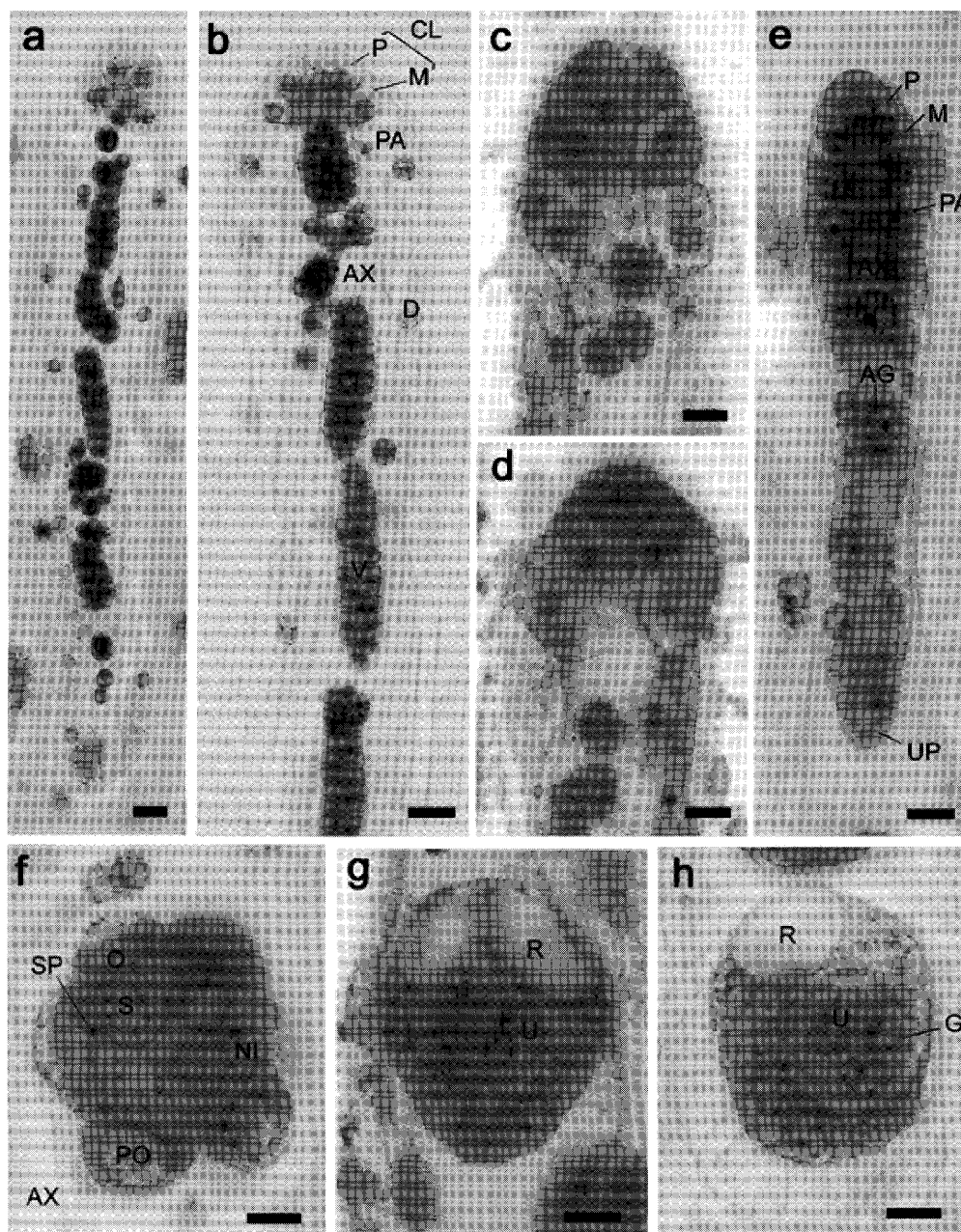


Fig. 7. *Dicyema awajiense* sp. nov. Photographs from syntype specimens on slide OUM-ME-00008. **a**, nematogen, entire; **b**, nematogen, anterior region; **c**, **d**, rhombogen, anterior region; **e**, vermiform embryo within axial cell; **f**, infusorigen; **g**, **h**, infusoriform embryos; **g**, horizontal section; **h**, sagittal section. Scale bars represent 10 μ m for **a**, **b**, 5 μ m for **c**–**h**. AG, agamete (axoblast); AX, axial cell; CL, calotte; D, diapolar cell; G, germinal cell; NI, nucleus of axial cell of infusorigen; M, metapolar cell; O, oogonium; P, propolar cell; PA, parapolar cell; PO, primary oocyte; R, refringent body; S, spermatogonium; SP, sperm; U, urn cell; UP, uropolar cells; V, vermiform embryo.

Off Akashi, 34°37'N, 135°02'E. Date of collection, 26 January 2005.

Etymology. The specific name refers to Awaji Island, near the type locality.

Symbiotype. *Amphioctopus fangsiao* (Cephalopoda: Octopoda: Octopodidae), mature female, 61 mm ML. OUM-MO-00007 (author's code FA1260)

Site of infection. Within renal sacs; anterior ends (calottes) inserted into crypts of the renal appendages.

Incidence. Dicyemids found in 7 host octopuses obtained in Harima Nada and Osaka Bay: 1.4% occurrence

among 507 octopuses examined.

Distribution. Known only from the type locality.

Description. *Nematogens* (Figs. 7a, b; 8a, c): Body slender; length ranging from 200 to 400 μ m, width from 30 to 50 μ m. Peripheral cell number 22 (Table 3): 4 propolars, 4 metapolars, 2 parapolars, 10 diapolars, and 2 uropolars. Calotte bluntly rounded, conical (Figs. 7a, b; 8c). Cilia on calotte short, about 5 μ m long, oriented forward. Cytoplasm of both propolar and metapolar cells stained with hematoxylin. Propolar cells and their nuclei smaller than metapolar cells and their nuclei, respectively. Trunk mostly uniform in

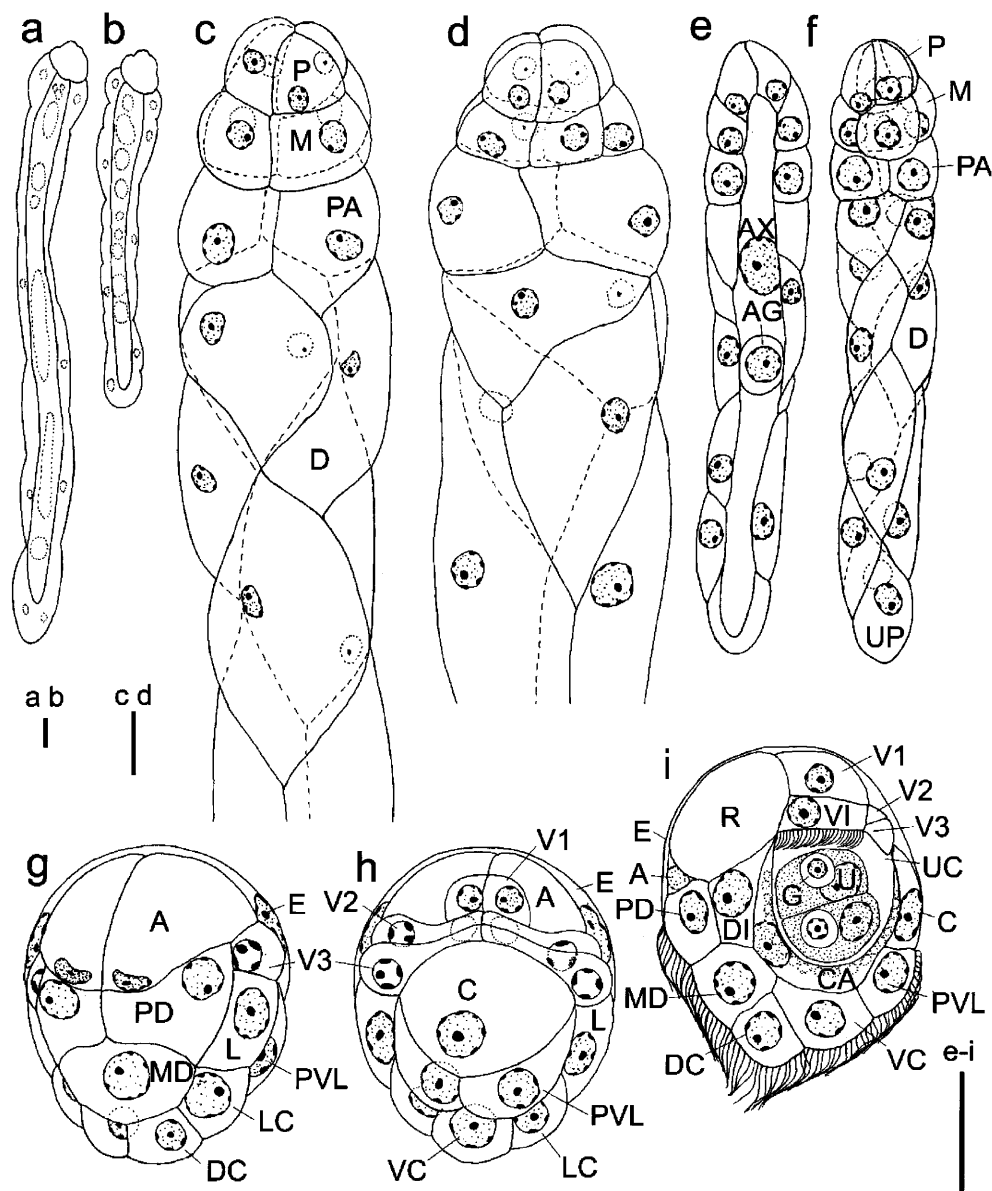


Fig. 8. *Dicyema awajiense* sp. nov. Drawn from syntype specimens on slide OUM-ME-00008. **a**, nematogen, entire; **b**, rhombogen, entire; **c**, nematogen, anterior region; **d**, rhombogen, anterior region; **e**, **f**, vermiform embryos within the axial cell; **e**, optical section; **g**–**i**, infusoriform embryos; **g**, dorsal view (cilia omitted); **h**, ventral view (cilia omitted); **i**, sagittal section. A, apical cell; AG, agamete (axoblast); AX, axial cell; C, couvercle cell; CA, capsule cell; D, diapolar cell; DC, dorsal caudal cell; DI, dorsal internal cell; E, enveloping cell; G, germinal cell; L, lateral cell; LC, lateral caudal cell; M, metapolar cell; MD, median dorsal cell; P, propolar cell; PA, parapolar cell; PD, paired dorsal cell; PVL, poster-oventral lateral cell; R, refringent body; U, urn cell; UC, urn cavity; UP, uropolar cells; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V2, second ventral cell; V3, third ventral cell.

Table 3. *Dicyema awajiense* sp. nov.: number of peripheral cells.

Cell number	Number of individuals		
	Vermiform embryos	nematogens	Rhombogens
22	50	50	50

width. Trunk cells arranged in opposed pairs. Axial cell cylindrical, rounded anteriorly; extending forward to base of propolar cells (Fig. 7b). In axial cell of large individuals, 5–10 vermiform embryos present. Accessory nuclei seen in

peripheral trunk cells.

Vermiform embryos (Figs. 7e; 8e, f): Full-grown vermiform embryos small-sized; length ranging from 40 to 70 μm , width from 8 to 11 μm ; peripheral cell number 22 (Table 3). Anterior end of calotte tapered anteriorly, bluntly rounded. Trunk cells arranged in opposed pairs. Axial cell rounded anteriorly; extends forward to middle of propolar cells (Figs. 7e; 8e). Axial cell nucleus typically located in center of axial cell. Axial cell of full-grown embryos containing single agamete.

Rhombogens (Figs. 7c, d; 8b, d): Slightly stockier than nematogens, otherwise generally similar in shape and body

proportions; length ranges from 200 to 400 μm ; width from 30 to 50 μm . Peripheral cell number 22 (Table 3). Calotte conical, as in nematogens. Shape and anterior extent of axial cell similar to those of nematogens. A single infusorigen present in axial cell. In the axial cell of large individuals, 10–15 infusoriform embryos typically present. Accessory nuclei present in peripheral cells.

Infusorigens (Fig. 7f): Small-sized. Axial cell of infusorigens usually rounded, diameter 8–10 μm . In mature infusorigens ($n=20$), number of external cells (oogonia and primary oocytes) 4–12 (mode, 8), number of internal cells (spermatogonia, primary spermatocytes, and secondary spermatocytes) 3–6 (mode, 4), and number of sperm 4–12 (mode, 6). Diameter of fertilized eggs 10.4 μm ; diameter of sperm 1.3 μm .

Infusoriform embryos (Figs. 7g, h; 8g–i): Ovoid, bluntly pointed posteriorly. In full-grown embryos ($n=50$), length (excluding cilia) 18.0 ± 1.2 μm (mean \pm S.D.) length-width-height ratio 1:0.88:0.82. Cilia at posterior end, 5.1 μm long. Refracting bodies present, solid, occupying anterior 40% of embryo length when viewed laterally (Fig. 7h). Cilia projecting from ventral internal cells into urn cavity (Fig. 8i). Cytoplasm of dorsal internal cells transparent. Capsule cells with many large granular inclusions. Full-grown infusoriform embryos ($n=50$) consisting of 37 cells: 33 somatic and 4 germinal cells. Somatic cells of several types: external cells covering large part of anterior and lateral surfaces of embryo (2 enveloping cells); external cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, 2 posteroventral lateral cells); external cells with refringent bodies (2 apical cells); external cells without cilia (1 couvercle cell, 2 first ventral cells, 2 second ventral cells, 2 third ventral cells); internal cells with cilia (2 ventral internal cells); and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, 4 urn cells). Each urn cell containing one germinal cell and one nucleus (Fig. 8i). Nuclei of third ventral cells pycnotic. All somatic nuclei typically become pycnotic as infusoriform embryos mature.

Remarks. *Dicyema awajiense* sp. nov. has been found

together with *D. akashiense*, *D. erythrum*, *D. colurum*, and *D. helocephalum* in *Amphioctopus fangsiao*. It is easily distinguishable from *D. colurum* and *D. helocephalum* by calotte shape (cone-shaped vs. disc-shaped). It is also easily distinguished from *D. akashiense* and *D. erythrum* by the number of peripheral cells of vermiform stages (22 vs. 15–17 and 32–36, respectively).

Dicyema misakiense is very similar to *D. awajiense* in calotte shape, number of peripheral cells in vermiform stages, and cell number of infusoriform embryos. However, *D. misakiense* differs from *D. awajiense* in having anterior lateral cells in infusoriform embryos instead of third ventral cells, and in having a much larger body size of full-grown vermiform stages (1800 μm vs. 400 μm).

Dicyema awajiense is similar to *D. aegira* McConnaughey and Kritzler, 1952, *D. acciacatum* McConnaughey, 1949, and *D. briarei* Short, 1961 in calotte shape and the number of peripheral cells in vermiform stages. However, *D. awajiense* differs from these three species in the anterior extent of the axial cell in vermiform stages (extending to propolars vs. to base of metapolars).

Dicyemid fauna in *Amphioctopus fangsiao*

Five dicyemid species were found in 506 individuals of *Amphioctopus fangsiao* from Osaka Bay (Table 4). Several different patterns were observed in *A. fangsiao* in the complement of dicyemid species occurring in octopus individuals and in the renal organs on opposite sides (Tables 4, 5). Never were all five species simultaneously present (Table 4). From one to four dicyemid species were observed in a single octopus individual, but in no case did four dicyemid species did co-occur in a single renal sac (Table 5). In two octopus individuals, a single dicyemid species was found only on the right side. *Dicyema erythrum* was the most commonly found species, with an occurrence of 81%. In contrast, the occurrence of *Dicyema colurum* and *D. awajiense* was only 0.4% and 1.4%, respectively.

In 40 octopus individuals (8%), the species composition of dicyemids differed between the renal organs on the right and left sides, with 14 different occurrence patterns (Table

Table 4. Dicyemid species found in 507 octopus individuals.

Dicyemids	Number of octopus individuals
<i>D. akashiense</i>	77 (15.2%)
<i>D. erythrum</i>	363 (71.6%)
<i>D. helocephalum</i>	7 (1.4%)
<i>D. akashiense</i> + <i>D. awajiense</i>	2 (0.4%)
<i>D. akashiense</i> + <i>D. colurum</i>	1 (0.2%)
<i>D. akashiense</i> + <i>D. erythrum</i>	28 (5.5%)
<i>D. akashiense</i> + <i>D. helocephalum</i>	6 (1.0%)
<i>D. colurum</i> + <i>D. erythrum</i>	1 (0.2%)
<i>D. erythrum</i> + <i>D. helocephalum</i>	11 (2.2%)
<i>D. akashiense</i> + <i>D. awajiense</i> + <i>D. helocephalum</i>	4 (0.8%)
<i>D. akashiense</i> + <i>D. erythrum</i> + <i>D. helocephalum</i>	5 (1.0%)
<i>D. akashiense</i> + <i>D. awajiense</i> + <i>D. erythrum</i> + <i>D. helocephalum</i>	1 (0.2%)
None	1 (0.2%)

5). Most (466, or 92.1%) octopus individuals had the same dicyemid species composition on opposite sides, in eight different occurrence patterns; 438 of these octopus individuals harbored only a single dicyemid species, either *D. akashiense* or *D. erythrum*.

Calotte shapes and co-occurrence patterns

There was some diversity in calotte shape among dicyemids examined in *A. fangsiao*, with three principal types observed: conical, cap-shaped, and discoidal (Fig. 9). Following Furuya *et al.* (2003), dicyemids with a conical (cone- or bullet-shaped) calotte are termed Type I. In this type, the anterior end can be either bluntly rounded or acutely pointed. Type I is further subdivided by the relationship of calotte length to calotte width. In Type Ia (e.g., *D. akashiense*), calotte length is greater than width. In Type Ib (e.g., *D. awajiense* and *D. erythrum*), length equals width. In Type II (e.g., *D. colurum*), the calotte is bluntly rounded (cap- or dome-shaped), and length is shorter than width. In Type III (e.g., *D. helocephalum*), the calotte is flattened and discoidal (disc-shaped); the propolar cells are engulfed laterally by the metapolar cells; and the parapolar cells together with the calotte constitute a cephalic swelling.

In *A. fangsiao*, 46 renal organs from 32 host individuals contained two or three dicyemid species (Table 6). Two dicyemid species co-occurred in 34 renal organs (26 host

individuals). Three dicyemid species coexisted in 12 renal organs (6 host individuals). In these cases, the calottes of co-occurring dicyemids were of different types.

Infusorigen size and number

The size and number of infusorigens are characteristic of dicyemid species (Furuya *et al.*, 1993). There is a negative curvilinear relationship between the number of infusorigens per rhombogen and the number of gametes per infusorigen (Furuya *et al.*, 2003b). Two distinct groups of dicyemid species are apparent. One type forms a small number of infusorigens, each with a relatively large number of gametes (4 to 70), as in *D. erythrum* and *D. colurum*. The other type tends to produce a large number of infusorigens, each with at most 20 gametes, as in *D. orientale*. Rhombogens of *D. acuticephalum* have a relatively small number of small-sized infusorigens with few gametes, and thus this species does not belong to either of these two types. Species in which the rhombogen produces a small number of infusorigens, with each infusorigen containing a small number of gametes, are, in fact, not rare (Furuya *et al.*, 2003b). Infusorigens of *D. awajiense*, *D. akashiense*, and *D. helocephalum* belong to this type. The bodies of these species, especially *D. awajiense*, are small, as in other exceptional species. These species have probably evolved through the process of progenesis.

Table 5. Combination of dicyemid species found in the left and right renal organs of individual octopus specimens (n=507).

Left	Right	Number of octopus individuals
<i>D. akashiense</i>	<i>D. erythrum</i>	16 (3.2%)
<i>D. akashiense</i>	<i>D. helocephalum</i>	3 (0.6%)
<i>D. awajiense</i>	<i>D. akashiense</i>	2 (0.4%)
<i>D. colurum</i>	<i>D. erythrum</i>	1 (0.2%)
<i>D. colurum</i>	<i>D. akashiense</i> + <i>D. colurum</i>	1 (0.2%)
<i>D. erythrum</i>	<i>D. akashiense</i>	7 (1.4%)
<i>D. helocephalum</i>	<i>D. akashiense</i>	2 (0.4%)
<i>D. helocephalum</i>	<i>D. erythrum</i>	2 (0.4%)
<i>D. akashiense</i> + <i>D. erythrum</i>	<i>D. awajiense</i> + <i>D. helocephalum</i>	1 (0.2%)
<i>D. awajiense</i> + <i>D. helocephalum</i>	<i>D. akashiense</i>	1 (0.2%)
<i>D. erythrum</i> + <i>D. helocephalum</i>	<i>D. akashiense</i>	1 (0.2%)
<i>D. akashiense</i> + <i>D. helocephalum</i>	<i>D. erythrum</i>	1 (0.2%)
None	<i>D. akashiense</i>	1 (0.2%)
None	<i>D. erythrum</i>	1 (0.2%)
<i>D. akashiense</i>		76 (15.0%)
<i>D. erythrum</i>		362 (71.4%)
<i>D. helocephalum</i>		7 (1.4%)
<i>D. akashiense</i> + <i>D. erythrum</i>	Same species as in left side	5 (1.0%)
<i>D. erythrum</i> + <i>D. helocephalum</i>		9 (1.8%)
<i>D. akashiense</i> + <i>D. helocephalum</i>		1 (0.2%)
<i>D. akashiense</i> + <i>D. awajiense</i> + <i>D. helocephalum</i>		3 (0.6%)
<i>D. akashiense</i> + <i>D. erythrum</i> + <i>D. helocephalum</i>		3 (0.6%)
None	None	1 (0.2%)

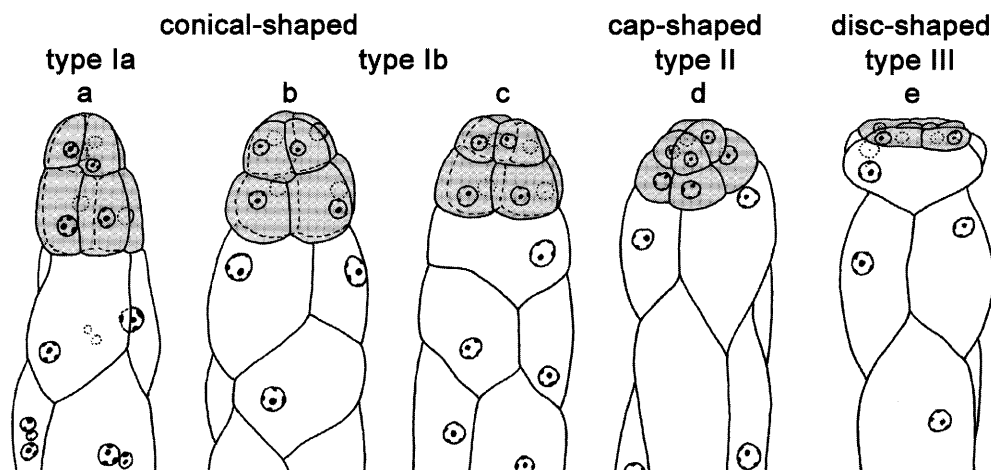


Fig. 9. Calotte shapes of dicyemid species in *Amphioctopus fangsiao*. **a**, *Dicyema akashiense*; **b**, *D. erythrum*; **c**, *D. awajiense*; **d**, *D. colurum*; **e**, *D. helocephalum*. See text for an explanation of each type.

Table 6. Co-occurrence patterns in a single renal sac of octopus individuals (n=46 renal sacs).

Dicyemids	Calotte types	Number of renal sacs
<i>D. akashiense</i> + <i>D. colurum</i>	Ia + II	1 (0.1%)
<i>D. akashiense</i> + <i>D. erythrum</i>	Ia + Ib	6 (0.6%)
<i>D. akashiense</i> + <i>D. helocephalum</i>	Ia + III	3 (0.3%)
<i>D. akashiense</i> + <i>D. awajiense</i>	Ia + Ib	2 (0.2%)
<i>D. awajiense</i> + <i>D. helocephalum</i>	Ib + III	2 (0.2%)
<i>D. erythrum</i> + <i>D. helocephalum</i>	Ib + III	20 (2.0%)
<i>D. akashiense</i> + <i>D. awajiense</i> + <i>D. helocephalum</i>	Ia + Ib + III	6 (0.6%)
<i>D. akashiense</i> + <i>D. erythrum</i> + <i>D. helocephalum</i>	Ia + Ib + III	6 (0.6%)

DISCUSSION

Dicyemid species in *Amphioctopus*

The genus *Amphioctopus* Fisher, 1882, was resurrected by Gleadall (2002) and Gleadall and Salcedo-Vargas (2004), and recently reviewed by Huffard and Hochberg (2005). This octopus group consists of small- to medium-sized octopuses living primarily on sand or mud substrates in tropical and sub-tropical waters. *Amphioctopus fangsiao* inhabits inshore waters and is widely distributed throughout the West Pacific from Japan to Hong Kong. In Japan, this species is abundant in February and March in the Seto Inland Sea area, and spawns around May (Gleadall and Naggs, 1991). Dicyemids have been recorded only from Japanese specimens of *A. fangsiao*. Several dicyemid species have been described from other species of *Amphioctopus*, including *A. areolatus* and *A. kagoshimensis* in Japanese waters (Furuya and Tsuneki, 2003; Furuya, 2005) and *A. burryi* (Voss, 1950) in the Gulf of Mexico (Furuya *et al.*, 2002). However, no dicyemids have been found in an undescribed *Amphioctopus* from Hawaii (Hochberg, pers. com.). This undescribed octopus inhabits holes or gaps among rocks or coral. Dicyemids may not infect cephalopods that live among corals and rocks, even though they are benthonic in habit (Furuya *et al.*, 2004b; but see Finn *et al.* [2006]).

Prevalence of dicyemids in *A. fangsiao*

In this study, five dicyemid species (*Dicyema akashiense*, *D. awajiense*, *D. colurum*, *D. erythrum*, and *D. helocephalum*) were found, with dicyemids occurring in 506 out of 507 individuals of *A. fangsiao* caught in Harima Nada and Osaka Bay; the prevalence of dicyemids thus approached 100%. Small or young cephalopods generally do not harbor dicyemids (Nouvel, 1937; Furuya *et al.*, 1992a), and in *O. vulgaris*, there is a direct relationship between host size and dicyemid occurrence (Furuya *et al.*, 1992a). In *A. fangsiao*, however, the individual harboring no dicyemids was of medium size (34 mm mantle length), whereas an individual of 28 mm mantle length harbored dicyemids, so the absence of dicyemids may not be attributable to the size of the host individual in *A. fangsiao*. Similar findings have been observed for *Amphioctopus kagoshimensis* (Furuya, 2005). Small-sized octopus species thus may be an exception to the relationship documented in *O. vulgaris*.

In *O. vulgaris*, dicyemids were rarely found in the renal organ on only one side (Furuya *et al.*, 1992a), but in *A. fangsiao*, various occurrence patterns of dicyemid species were observed. Dicyemids were found only in the right renal organ in two individuals. In one host individual, the two dicyemid species in one renal sac differed from the two species in the other sac (Table 5). This suggests that dicyemids infecting one side of the renal organ do not move to the

other side, and that dicyemids infect each renal organ independently. There is no direct connection between the two renal sacs. Furthermore, a renal organ typically harbors a great number of dicyemid individuals. If dicyemids could move from one side to the other, the same species should be found on both sides, and the various occurrence patterns observed in this study would not have been observed. The mode of infection is unknown.

Typically two to three, or occasionally four, species of dicyemids are found together in a single host individual. In the absence of interspecific competition, every possible combination of dicyemids would be present, and the prevalence of each dicyemid species would not be expected to differ significantly. However, the actual prevalence of each dicyemid species and the patterns of co-occurrence in each host species are very different from what has been predicted (Furuya *et al.*, 2003a). In most cases where four or five dicyemid species have been found to co-occur, only a single dicyemid species is predominant. For instance, of the five species found in *S. esculenta*, *P. nakaoi* Furuya, 1999 had the highest prevalence (61.0%). Likewise, in *A. fangsiao*, nearly 95% of renal organs contained a single dicyemid species, usually *D. erythrum*. Less than 5% of renal organs harbored two or three dicyemid species. The dominant species in a particular host possibly shows highest prevalence because it is best adapted to the habitat provided by that host.

Niche separation and convergence of calotte shapes

When two dicyemid species with distinct calotte shapes coexisted in the renal organ, dicyemids with a conical calotte (*e.g.*, *D. akashiense*, *D. awajense*, and *D. erythrum*) were found within the convolutions or crypts of the renal tissues, whereas those with a discoidal calotte (*e.g.*, *D. helocephalum*) attached to the surface of the renal tissues. This indicated that niche separation possibly occurs between these dicyemids. When dicyemids having a similar conical calotte shape (*e.g.*, *D. akashiense*, *D. awajense*, and *D. erythrum*) coexisted in a single renal organ, *D. erythrum* occurred in higher numbers than *D. akashiense* or *D. awajense*.

Similar patterns in the calotte morphologies of co-existing dicyemid species are found in the renal organs of various cephalopod species (Furuya *et al.*, 2003a). Calottes typically are conical in shape in the vermiform embryos of almost all species and in the adult vermiform stages in the majority of species. A conical calotte is thus the most commonly observed morphology in dicyemids. It is formed simply by proportional cell enlargement. In the process of ontogenetic growth from embryo to adult, the shape of the calotte changes from conical to cap-shaped to discoidal (Furuya *et al.*, 2003a). The cap-shaped calotte appears to be intermediate between what might be termed the plesiomorphic or primitive condition (conical calotte configuration) and a more advanced or apomorphic discoidal configuration. In this developmental sequence, a disc-shaped calotte represents a hypermorphic state. One mechanism by which various morphological characters evolved to reduce competition in each different host species might have been heterochrony.

The developmental patterns and cell lineages of vermiform embryos are highly conserved, and the morphology of embryos is little modified, among dicyemids found in differ-

ent cephalopod host species having different geographical distributions (both horizontal and vertical) (Furuya *et al.*, 2001). Such conservative developmental features can be attributed to the seemingly uniform microhabitat present inside the axial cells of the parent nematogens. Morphological differences principally evolve in response to selective pressures in the renal sac following release of embryos from the constraints of the restrictive microhabitat found in the axial cell of the parent. That is, it is after eclosion that dicyemids might evolutionarily adapt to the surface structure of renal organs. Calotte morphology possibly involves adaptation to the structure of the host renal organs and helps to facilitate niche separation of coexisting species.

Chromidina, a ciliate inhabitant of the renal organs of oceanic squids and cuttlefishes, exhibits morphologies of the attachment end that mirror shapes observed in the dicyemids (Hochberg, 1982, 1990; Furuya *et al.*, 2004b). The attachment regions of *Chromidina elegans* and *C. coronata* are comparable to dicyemid calotte shapes Type I and Type III, respectively. This is an obvious example of morphological convergence in two different groups of parasites that live exclusively in the renal organs of cephalopods.

Relationships among species and host specificity

Nouvel (1947) noted the presence of *D. misakiense* in *A. fangsiao*. Using adult characters only, he probably misidentified *D. awajense* n. sp. as *D. misakiense*. These two species cannot be distinguished by characters of the adult stages. Because Nouvel and Nakao (1938) did not separate *D. japonicum* Furuya and Tsuneki, 1992 from *D. misakiense*, Nouvel (1947) might have observed *D. helocephalum* n. sp., which is very similar to *D. japonicum*. These two species also cannot be separated by the characters of adult stages alone. The total cell number of infusoriform embryos in *D. awajense* and *D. helocephalum* is 37, as it is also in *D. misakiense* and *D. japonicum*. However, *D. awajense* is separable from *D. misakiense*, and *D. helocephalum* from *D. japonicum*, by cellular composition. This study also revealed a difference between *D. awajense* and *D. japonicum* in the cellular composition of infusoriform embryos. A total of 29 cells (15 cell types) is consistently recognized in the infusoriform embryos of 64 dicyemid species (Furuya *et al.*, 2004a). Even in infusoriform embryos with the same total cell numbers, cellular composition varies by species; for example, there are seven different cellular compositions in infusoriform embryos with 37 cells. The cellular composition of infusoriform embryos appears to be species-specific, and thus may include significant characters to be used in the identification and classification of dicyemids.

Most dicyemid species are host-specific. However, 18 dicyemid species are known to have a relatively wide host range (see Table 17 in Furuya [1999]). For instance, *Dicyema macrocephalum* van Beneden, 1876, appears to infect five cephalopod species belonging to three genera. The present study excludes three dicyemid species, *D. acuticephalum*, *D. japonicum*, and *D. misakiense*, from the list of non-host-specific species. The other species with a wide host range have been described mostly without using characters of the infusoriform embryos, so it will be necessary to examine the cellular composition of the infusoriform embryos of these other species to confirm their occurrence

in more than one species.

The dicyemid species of *A. fangsiao* are apparently similar to those of *A. kagoshimensis* and *O. vulgaris*. These three host cephalopods are closely related (Takumiya *et al.*, 2005). Resemblance in calotte shapes is possibly due to convergence. However, other similarities among dicyemids in these host species presumably reflect their presence in the closely related host species with which they have co-evolved.

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